Effect of alcoholic extract of aloe vera plant on serum testosterone and gonadotropin levels in rats

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Received: 01/01/2011 Revised: 03/12/2012 Accepted: 04/20/2012

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Journal of Jahrom University of Medical Sciences, Vol. 10, No. 2, Summer 2012

Abstract

Introduction:
Aloe vera plant’s beneficial effect on diabetes, burns, wounds, and gastrointestinal diseases has been proved. In this study, the effect of aloe vera extract on serum testosterone and gonadotropin levels was investigated in rats.

Materials and Methods:
In this experimental study, 45 adult male Westar rats with the average weight of 200 ± 20 g were randomly divided into 5 groups, including control, sham and experimental groups 1, 2, 3, which received orally 50, 100, 200 mg/kg bw aloe vera alcoholic extract per day respectively for 30 days. The rats were anaesthetized with ether, and blood was taken from the heart. Serum was separated and serum level of hormones LH, FSH and testosterone were measured by radioimmunoassay. The data were analyzed using ANOVA.

Results:
Results showed that serum levels of testosterone in the experimental groups 2 (100 mg) and 3 (200 mg) compared to the control group decreased significantly (P<0.05). Also, serum levels of LH and FSH hormones in the experimental group decreased as compared with the control group.

Conclusion:
According to the results of this study, aloe vera plant’s alcoholic extract taken orally can decrease testosterone and gonadotropin secretion in the male rats.

Keywords: Aloe Vera, Testosterone, Gonadotropin, Plants, Extracts

Introduction
Rapid growth of population poses a threat in the modern world, especially in developing countries. Given the limited amount of resources, the population cannot grow at the current pace for a long time. On the other hand, reducing the number of unplanned pregnancies may have a positive effect on welfare, population growth pattern and socioeconomic development of a country. Previous studies indicate that some 65% of unplanned pregnancies may be blamed on failure of contraception techniques [1]. In Iran, 18% of pregnancies are unwanted and modern contraceptive methods do not have an
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appropriate coverage. In a physically and mentally sane society, individuals are the primary decision makers of pregnancy and contraceptive techniques must be fully successful. Illegal abortions constitute another adverse complication of unplanned pregnancies. Each year, about 68 thousand women lose their life to unsafe abortion in developing countries [2]. Considering the lower risks associated with herbal medications compared to their chemically synthesized counterparts, it seems reasonable to identify and promote the use of medicinal plants.

Aloe vera belongs to the order of Aspargales, family of Xanthorrhoeaceae and subfamily of Asphodeloideae. It has over 250 known species worldwide. Botanically, it is a succulent plant with spear-shaped, serrated leaves. It usually grows to be 60 cm tall with a short wooden stalk. The leaves, which attach directly to the stem, are fleshy with a convex external surface and slightly concave internal surface. The flowers are yellow and form a closely packed cluster [3]. The plant blossoms in fall. Aloe vera has been used in traditional medicine for thousands of years, used in treatment of skin conditions and other diseases. The most important compounds include anthraquinones (aloein, aloe-emodin, coumaric acid), polysaccharides, glycoproteins, prostaglandins, cholesterol, and fatty acids such as campesterol [4-6].

Previous studies suggest antitoxic [7,8], antitumoral, antimicrobial, and antioxidant properties [9-13] for aloe vera. Moreover, is improves wound healing and activity of macrophages [14, 15]. One study dealing with aloe vera effects on ovaries of pregnant rats indicated that the plant increases the weight and improves vascularization around secondary follicles in rats. Moreover, it suggested the effects of aloe vera to be similar to those of estrogen and follicle stimulating hormone [16].

A recent study dealing with the impact of aloe vera on testosterone and gonadotropins in adult male rats indicated that the hydroalcoholic extract has antiandrogenic properties and may reduce androgen-related parameters, including tropin secretion, probably causing oligospermia [17]. Considering the little data regarding the impact of aloe vera and its compounds (such as aloe-emodin and coumaric acid) on the reproductive system, it is possible that these compounds may influence sex hormones. Therefore, we undertook the present study to investigate the impact of alcoholic extract of aloe vera leaf on testosterone and gonadotropin in male, adult rats.

Materials and Methods:
In this study, we used 45 adult, male Wistar rats. The mean weight of the rats on onset of the study was 200 ± 20 g and they were aged 2-3 months. The ambience temperature was kept constantly at 25 ± 2 °C throughout the study, and the rats had unlimited access to compact food. The rats were divided into 5 groups (each comprising 9 rats) in the following manner:

Control group: This group used water and compact food ad libitum throughout the study without any limitation.

Sham group: This group had unlimited access to food and water throughout the study, but received 2mm distilled water orally simultaneously and similar to experimental groups.

Experimental groups: Three experimental groups were respectively treated with 50, 100, and 200 mg/Kg body weight of aloe vera extract via oral route. The treatment continued for 30 days. At the end of the 30th day, the rats were anesthetized and blood samples were drawn from their ventricles. The samples were centrifuged at 3000 rpm for 10 min to yield serums. Hormone quantification was accomplished using radioimmunoassay.

The inclusion criteria were healthy male, adult Wistar rats weighing 200 ± 20 g and aged 2-3 months. Rats outside the age and
weight range were excluded from the study.

**Preparation of aloe vera extract:**
The plant extract was prepared using the standard soaking and percolation protocol described by Fariba Abolhasantash [21]. For this purpose, 500 g fresh, chopped leaves of the plant was soaked for 48 hours in a vessel containing 96% ethanol. The filtered solution was then centrifuged at 3000 rpm for 5 minutes to ensure the removal of suspending particles. The powder was prepared using a desiccator. The resulting dry powder was dissolved in distilled water at different amounts of 50, 100, and 200 mg. The volume of distilled water was 2mL for all doses of powder.

**Hormone quantification:**
Levels of testosterone and gonadotropins (follicle stimulating, follicle releasing) were determined through radioimmunoassay. For this purpose, blood serum containing unlabeled antigen is mixed with antigen labeled with iodine 125. Both antigens compete for binding to the standard labeled antibody which is added to the solution. The antibody is initially bound to the unlabeled antigen and the rest will bind with the labeled antigen. The supernatant, containing free labeled antigen and bound unlabeled antigen is disposed and only the labeled antigen bound with the antibody remains as deposit, which is then evaluated with a gamma counter.

**Data analysis:**
Data were analyzed on SPSS software using one-way analysis of variance. P values < 0.05 were considered significant.

**Results**
The following tables and diagrams present the results of comparing testosterone and gonadotropin levels among control, sham and experimental groups.

Our findings indicate that the serum level of testosterone in the experimental groups 2 and 3 was significantly lowered compared to the control group (p<0.05). In the first experimental group, however, the serum level of testosterone was not different from the control group (Diagram 1).

As for the follicle releasing hormone, our results indicate that the serum levels of this hormone was reduced at doses of 50, 100, and 200 mg/Kg aloe vera alcoholic extract; the difference, however, was not significant between the experimental, control and sham groups (p<0.05) (Diagram 2).

All three doses of aloe vera extract reduced the serum levels of follicle stimulating hormone; nevertheless, the reduction was not significant between experimental, control and sham groups (p<0.05) (Diagram 3).
Effect of alcoholic extract of aloe vera on mean serum level of testosterone, luteinizing hormone, and follicle stimulating hormone among control, sham, and experimental groups of male rats at the end of 30th day.

Diagram 1: Comparing the effect of different concentrations of aloe vera alcoholic extract on mean serum level of testosterone among control, sham, and experimental groups of male rats at the end of 30th day.

Diagram 2: Comparing the effect of different concentrations of aloe vera alcoholic extract on mean serum level of luteinizing hormone among control, sham, and experimental groups of male rats at the end of 30th day.

Diagram 3: Comparing the effect of different concentrations of aloe vera alcoholic extract on mean serum level of follicle stimulating hormone among control, sham, and experimental groups of male rats at the end of 30th day.

Table 1: Comparing the mean serum levels of testosterone and gonadotropins among groups treated with different doses of aloe vera alcoholic extract and control in male adult rats at the end of 30th day.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Testosterone (mg/dL)</th>
<th>Mean LH (mg/L)</th>
<th>Mean FSH (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.37 ± 4.37</td>
<td>0.37 ± 0.103</td>
<td>0.28 ± 0.192</td>
</tr>
<tr>
<td>Sham</td>
<td>5.35 ± 2.16</td>
<td>0.34 ± 0.11</td>
<td>0.27 ± 0.09</td>
</tr>
<tr>
<td>Dose 50</td>
<td>7.71 ± 6.18</td>
<td>0.27 ± 0.646</td>
<td>0.22 ± 0.119</td>
</tr>
<tr>
<td>Dose 100</td>
<td>1.39 ± 0.32*</td>
<td>0.23 ± 0.060</td>
<td>0.20 ± 0.173</td>
</tr>
<tr>
<td>Dose 200</td>
<td>2.62 ± 0.82*</td>
<td>0.22 ± 0.123</td>
<td>0.16 ± 0.188</td>
</tr>
</tbody>
</table>

* indicates significance compared to the control group (p<0.05)
**Discussion**

In the present study, we observed that aloe vera extract significantly reduced the level of testosterone at doses of 100 and 200 mg/Kg body weight (p<0.05) (Diagram 1). This is consistent with findings of Shariati et al [17]. Recent studies indicate that certain compounds in aloe vera, e.g. coumaric acid, may stimulate the activity of testicular macrophages which are responsible for nitrous oxide production, and suppress the conversion of cholesterol to pregnenolone through inhibition of p450 cytochrome activity, thus reducing testosterone secretion [18].

Studies conducted on the impact of phytoestrogens on testosterone in rats indicate that phytoestrogens lower the serum concentration of testosterone [19]. It is possible that aloe vera flavones exert their effect through binding with hormone receptors or hormone metabolizing enzymes, similar to other phytoestrogenic compounds [20]. On the other hand, it appears that the impact of flavonoids on hormone metabolizing enzymes, including type 5 human 17 β-hydroxysteroid hydrogenase is particularly important. This enzyme constitutes a link in the testosterone production pathway and is suppressed by phytoestrogens [21].

Considering the results of previous studies which suggest the presence of isoflavones in aloe vera, it seems that the lowering of testosterone level may be attributed to these phytoestrogenic compounds. Previous studies indicate that the compounds present in aloe vera interfere with androgen production and lower the levels of testosterone and follicular stimulating hormone [17].

Studies indicate that phytoestrogens affect the hypothalamus to suppress the production of gonadotropin releasing hormone, thus inhibiting the hypothalamus-pituitary-gonad axis [22]. The gonadotropin releasing hormone secreted from hypothalamus affects the anterior pituitary to stimulate the secretion of FSH and LH. The latter affects Sertoli cells to promote the production and secretion of androgen binding protein [17]. A reduction on androgen levels will suppress the activity of androgen binding protein, lowering the estradiol levels.

The findings of the present study indicate that aloe vera extract lowers the serum level of FSH in experimental groups, although the reduction was not significant (Diagram 3). Shariati et al demonstrated that the hydroalcoholic aloe vera extract at 100 and 150 mg/Kg body weight lowers the serum level of FSH significantly compared to the control group [17]. The discrepancy may be attributed to the difference in duration of treatment and type of extract. In the study mentioned above, the extract was administered for 21 days, while we used a treatment period of 30 days. On the other hand, we used the ready powder of the plant, while Shariati et al used aloe vera gel [17].

In the present study, serum levels of LH were lowered in the experimental group compared to the control group, albeit the difference was not significant (Diagram 2). We expected the levels of LH to rise via negative feedback due to testosterone reduction, however, the presence of active compounds in the extract, including aloe emodin, may have directly affected gonadotropins to reduce LH [20]. Other potential mechanisms include the downgrading of LH receptors or reduced sensitivity of these receptors. A reduction in the number of these receptors on Lydig cells in testes will compromise the synthesis and secretion of testosterone.

Shariati et al studied the impact of aloe vera extract on serum levels of LH, FSH and testosterone. They did not find a significant change in LH levels, which is in line with our findings [17]. It is possible that the feedback-based mechanism of LH secretion from pituitary in reaction to testosterone reduction requires a period...
longer than 30 days [17]. Roberts et al (2000) demonstrated a lowering of LH levels in rats exposed to genistein – a phytoestrogens [23]. McGrawe et al studied the impact of a diet rich in phytoestrogens on humans and animals to conclude that campesterol suppresses the secretion of LH, while genistein has no such effect [24]. Previous studies indicate that aloe vera increases the number of secondary follicles, reduces their size and improves their vascularity in the ovaries of pregnant rats. The development of antral (secondary) follicle is largely dependent on FSH and aloe vera exerts an effect similar to that of FSH in female rats. These are all similar to the effects of estrogen on genitalia [16]. Given the little data available regarding the biologic impacts of aloe vera extract on the reproductive system, it is extremely difficult to interpret its impact on testosterone and gonadotropin levels.

**Conclusion**

Considering the findings of the present study, the alcoholic extract of aloe vera generally reduces testosterone and gonadotropin levels in male rats. Further studies are recommended to address the possible applications of this plant in regulating fertility in the male sex.

**Acknowledgement**

The authors wish to express their gratitude to the officials at vivarium of Shiraz University of Medical Sciences, as well as Dr. Azarpira and Mr. Bazrafshan who collaborated in this study.

**References:**